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# Seed-Borne Fungi of Sunflower (*Helianthus annuus* L.) and their Impact on Oil Quality

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***Abstract:*** *The deteriorating in sunflower oil due to seed-borne fungi is of a great importance. In the present study ten seed-borne fungi were isolated from abnormal sunflower seeds collected from different locations in Egypt i.e. Aspergillus flavus, A. niger, Alternaria alternata, Curvularia lunata ,Fusarium moniliforme, F. oxysporum, F . semitectum, Penicillum digitatum, Stemphylium sp., and Trichoderma spp. A noticeable variation was recorded in sunflower oil samples such as chemical properties i.e. saponification number, fatty acid value, iodine number, peroxide value also, physical properties i.e. moisture content ,gravity, odor absorbent, absorbance (470nm) and oil colour ,these differences are due to the secondary metabolites produced by storage fungi. Some of the tested fungi gave a remarkable differences in both of absorbance and odor i.e. with F. semitectum and Stemphylium sp. treatments.*
***Keywords:*** *Sunflower seed-borne fungi, oil deterioration, chemical and physical properties, biochemical changes of oil samples.*

## I. Introduction:

Sunflower (*Helianthus annuus* L.), considered a commercial oil crop all over the world. The crop is widely cultivated in Egypt and all over the world. Sunflower is particularly used for production of edible oil as well as for seed consumption [1], [2] and [3]. Seeds used for cultivation of the crop are mostly hybrids in Egypt. However, during the last years some attempts were made to produce sunflower hybrids and new genotypes adapted to the climate of different regions in Egypt and to be higher in oil production. Sunflower is one of the most important producers of oil among different oil crops all over the world. Fats and oils are important ingredients of human food. Sunflower seeds contain 40-50% oil and 23% protein and constitute an excellent source of unsaturated fats, crude protein and fibers and important nutrients like many vitamins .Sunflower is affected by a large number of diseases caused by many fungi, and other phytopathogenic microorganisms. Most of sunflower fungal species are reported to be seed-borne [4]. Thirteen isolated phytopathogenic fungal species were isolated from different stored sunflower varieties .Externally seed-borne mycoflora are saprophytes but a few were parasites also occur along with them. The internal seed mycoflora is composed of parasites as well as saprophytic organisms. The presence of 13 species in unsterilized and 12 species from sterilized sunflower seeds was detected and reported by [5]. During storage conditions sunflower seeds are exposed to various infections by microorganisms like fungi which may lead to various damage including reducing yields of seed in both qualitatively and quantitatively, besides these decreases in germination percentage, mycotoxin production and total decay has been observed [4],[ 5].

The present study was carried out to survey seed-borne mycoflora of sunflower, testing the effect of some storage fungi on sunflower oil quality and to investigate some of the physical and the chemical properties of deteriorating oil.

## II. Materials and Methods:

**Source of seed samples:**

Ten sunflowers (*Helianthus annuus* L.); seed samples were collected from different locations in Assiut governorate in Egypt the obtained samples were brought to the laboratory and kept at room temperature for the present study as shown in Fig.(1).

**Isolation of sunflower seed- borne fungi:**

A subsample of 100 abnormal sunflower seeds from each sample was surface sterilized in 2.0% Clorox solution for one minute, and washed several times with sterilized water. The surface sterilized seeds were then blotted between two dry sterilized filter paper. Seed- borne fungi were determined by the standard PDA method [6] and [7] .Twenty five sterilized sunflower seeds of each sample were randomly selected and plated on Petri dishes, each was replicated 4 times; plates were incubated for 12-hour duration of darkness and light at 20± 2 $C^0$ for 7 days .After incubation period each colony examined macroscopically or microscopically for identification the genus or species level according to [6] and [7].

**Fig.(1).Showing a working sample of sunflower seeds submitted for isolation with the standard PDA method.**

**Properties of the sunflower oil samples:**

The refined sunflower oil samples were collected from the oil producers in sterilized tubes. These samples were brought into the laboratory. Spores and mycelium fragments of ten fungi each fungus individually which previously isolated from abnormal sunflower seeds were inoculated in a conical flask containing 100 ml oil each of sunflower oil samples (10 samples) under aseptic conditions. After thirty days later, oil was filtered and these oil samples were used for the estimation of both chemical and physical properties under sterilized conditions according the described method by [8].

**Physical properties:**

**1- Determination of moisture content from oil:**

The moisture content was estimated [9]. About 1ml of oil was taken in a moisture dish provided with tight filling slip over cover. The dish was dried previously, cooled in the desiccators (containing an efficient desiccant) and weighed. The dish was placed in the air oven for approximately two hours at 105 $C^0$. The dish was removed from the oven, cooled in the desiccators at room temperature and weighed. This procedure was repeated but the dish kept in the oven only for half an hour each time until the difference between the two successive weighing does not exceed one milligram. The moisture content was calculated by following formula:

$$\text{Moisture \%} = \frac{100\,[M1 - M2]}{MI - M}$$

M1 = mass in gm of the dish with the material before drying process.
M2 = mass in gm of the dish with the material after drying process.
M = mass in gm of the empty dish.

**2- Determination of oil colour:**

Colour of deteriorated oil was determined by observing the grade of the yellow colour as yellow, bright yellow, dark yellow, light yellow and pale yellow and so on.

**3- Determination of specific gravity:**

The specific gravity was estimated [8], [9]. The weight of dry specific gravity bottle was taken (B). The dry specific gravity bottle filled with the 5ml of the sample. After fixing the stopper, weight was taken (A). The weight of the specific gravity bottle containing 5ml of distilled water was taken (C). The specific gravity was calculated by the formula:

$$\text{Specific gravity at } 30\ C^0 = \frac{A - B}{C}$$

A=Weight in gm of specific gravity bottle with oil at 30 $C^0$.

B=Weight in gm of specific gravity bottle at 30 $C^0$.
C=Weight in gm of specific gravity bottle with distilled water at 30 $C^0$

**4- Determination odor of deteriorated oil:**

For this test the odor of the deteriorated seed oil was determined by just smelling the sample under the study to detect its case in relation to the oil in check treatment.

**5-Absorbance of deteriorated oil:**

The O.D. at 420nm absorbance of deteriorated oil was recorded to study the effect of storage fungi on the tested oil samples infected with target fungal individually.

**Chemical parameters:**

**1- Determination of peroxide value:**

The peroxide value of sunflower oil was calculated; Fig. (2). One gm of sample of oil was taken in a test tube 20 ml acetic acid / chloroform solution (2:3 volumes) and 1g powdered potassium iodide was added. The tube was placed in boiling water bath until liquid boil vigorously. The contents were quickly transferred to the flask containing 20ml of 5% KI solution. The tube was washed quickly with 25ml Distilled water each time and collected in a conical flask, yellow colour was appeared. This was then titrated with 0.1 N sodium thiosulphate solution with constant and vigorous shaking. The titration was continued till the yellow colour almost disappeared 0.5 ml of starch solution was added and continued titration till the blue colour just disappeared. A blank determination of reagent was conducted. Peroxide value was calculated by the formula:

$$\text{Peroxide value} = \frac{[S-B] \times N \times 100}{\text{Sample weight}}$$

B= Titration of blank test ml.
S= Titration of sample ml.
N= Normality of sodium thiosulphate solution

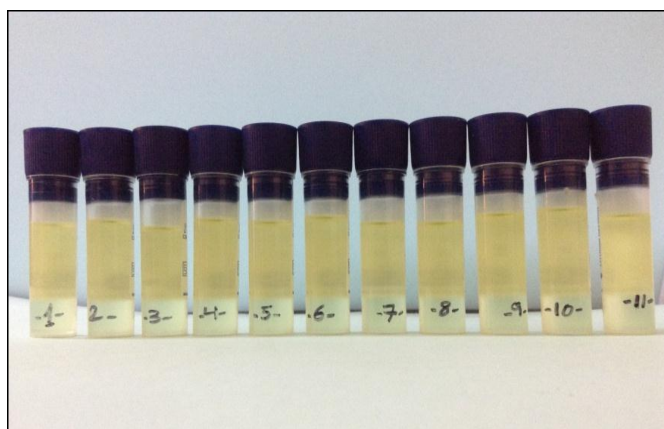


**Fig.(2). Showing a subsamples of sunflower oil used in the study where, no.11 is check treatment.**

**2- Determination of iodine value:**

The iodine value was determined according to the titrometric method as reported by [8]. 2g of oil sample was weighed into a dry glass bottle of 250 ml capacity and 10ml of carbon tetrachloride was added to the oil. U-boat 20ml of solution (Mix 1.5 % of Iodine monochloride and 97% of Glacial acetic acid) was then added and allowed to stand in the dark for 30 min. 15ml of (10%) potassium iodide and 100ml of water was added and then titrated with 0.1M sodium thiosulphate solution using starch as indicator just before the end point. A blank was also prepared alongside the oil samples. Iodine value was calculated from the formula:

$$\text{Iodine value} = \frac{(V_2 - V_1) \times 1.269}{\text{Sample Weight (g)}}$$

Where: V1=titer value for sample

V2= titer value for blank
S = sample weight in (g)

**3- Determination of Saponification value:**

The Saponification value was determined according to the titer metric method [9]. 2g of oil sample was weighed into a conical flask and 25ml of alcoholic potassium hydroxide was added. The solution was heated in boiling water for 1hour. 1ml of 1% phenolphthalein was added and titrated with 0.5N Hcl. A blank was prepared alongside the oil samples the value was calculated by the formula:

$$\text{Saponification no.} = \frac{56.1 \times (B-S) \times N}{\text{Sample Weight (g)}}$$

Where:

B=Volume in ml of 0.5 N Hydrochloric acid.
S=Volume in ml of 0.5 Hydrochloric acid.
N=Normality of Hydrochloric acid.
W=Weight of oil in gm

**4-Determination of free fatty acid content:**

Free fatty acid content was estimated by the method recommended [9]. 2ml of oil was dissolved in 50ml of neutral solvent in 250ml conical flask. Few drops of phenolphthalein indicator were added and titrated against 0.1N potassium hydroxide. Constant shaking was done until pink colour was persisted for fifteen seconds and acid value was calculated by the formula:

$$\text{Acid value} = \frac{\text{Titer value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of sample (g)}}$$

## III. Results and Discussion:

The data shown in Table (1) indicate that out of ten samples of sunflower seeds (*Helianthus annuus* L.), ten seed-borne fungi were detected *i.e. Aspergillus flavus, Aspergillus niger,Alternaria alternata, Curvularia lunata, Fusarium moniliforme, F.oxysporum, F. semitectum,Penicillim digitatum,Stemphylium* sp.and *Trichoderma* spp. ; the most dominant fungus was *Aspergillus niger* with all of the examined samples followed by *Aspergillus flavus* and *Fusarium* spp. The obtained data are similar to those reported by [1],[2] and [3], they stated that seed mycoflora are of a great importance for seed deterioration and consequently lead to seed losses and this may be as a result of the secretion of mycotoxin and fungal secondary metabolites which reduce seed quality and quantity as reported by [9], [10] and [11].

**Table (1).Percentage of seed infection using standard PDA method for ten sunflower seed samples.**

| Isolated fungi | *S-1 | S-2 | S-3 | S-4 | S-5 | S-6 | S-7 | S-8 | S-9 | S-10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Aspergillus flavus* | 12.0 | 10.0 | 12.0 | 16.0 | 18.0 | 17.0 | 19.0 | 19.0 | 10.0 | 5.0 |
| *Aspergillus niger* | 24.0 | 13.0 | 22.0 | 7.0 | 16.0 | 18.0 | 16.0 | 15.0 | 17.0 | 17.0 |
| *Alternaria alternata* | 30.0 | 21.0 | 17.0 | 18.0 | 0.0 | 0.0 | 13.0 | 21.0 | 18.0 | 15.0 |
| *Curvularia lunata* | 5.0 | 10.0 | 18.0 | 24.0 | 17.0 | 2.0 | 22.0 | 11.0 | 19.0 | 12.0 |
| *Fusarium moniliforme* | 7.0 | 19.0 | 2.0 | 0.0 | 0.0 | 15.0 | 12.0 | 13.0 | 10.0 | 14.0 |
| *Fusarium oxysporum* | 4.0 | 0.0 | 10.0 | 12.0 | 3.0 | 2.0 | 16.0 | 17.0 | 0.0 | 18.0 |
| *Fusarium semitectum* | 0.0 | 8.0 | 11.0 | 0.0 | 11.0 | 11.0 | 0.0 | 0.0 | 17.0 | 0.0 |
| *Penicillum digitatum* | 7.0 | 11.0 | 0.0 | 5.0 | 18.0 | 17.0 | 0.0 | 4.0 | 0.0 | 11.0 |
| *Stemphylium* sp. | 0.0 | 5.0 | 0.0 | 10.0 | 0.0 | 8.0 | 0.0 | 0.0 | 0.0 | 8.0 |
| *Trichoderma* spp. | 11.0 | 3.0 | 8.0 | 8.0 | 17.0 | 10.0 | 2.0 | 0.0 | 9.0 | 0.0 |

**Based on examination of 100 sunflower seeds with the standard PDA method.**
***S=Sample number**

Recorded data in Table (2) show the percentage of each individual isolated fungus from sunflower seeds. The highest percentage was noticed with *Aspergillus niger* ( 16.5%), followed by *Alternaria alternata* (15.3%) and *Aspergillus flavus* (13.8), respectively .On the other hand the lowest percentage was shown with *Stemphylium* sp.(3.1%). The obtained results are somewhat similar to those reported by [4] and [5]. In their scientific work they reported that, the association and isolation frequencies of some fungal species isolated from sunflower seeds were clearly correlated with seeds deterioration and decay some factors may indicate the

presence of a mixed relationships between sunflower seed-borne fungi and the extracted oil seed quality which affects on sunflower oil crop production.

**Table (2). Mean percentage of seed infection using standard PDA method for each isolated fungi.**

| Isolated fungi | Mean percentage (%) |
|---|---|
| *Aspergillus flavus* | 13.8 |
| *Aspergillus niger* | 16.5 |
| *Alternaria alternata* | 15.3 |
| *Curvularia lunata* | 14.0 |
| *Fusarium moniliform*e | 9.2 |
| *Fusarium oxysporum* | 8.2 |
| *Fusarium semitectum* | 5.8 |
| *Penicillum digitatum* | 7.3 |
| *Stemphylium* sp. | 3.1 |
| *Trichoderma* spp. | 6.8 |

**Data recorded as mean ( % ) of ten sunflower seed samples .**

Concerning the physical changes in sunflower oil due to the deterioration process of oil data in Table (3) show oil colour, absorbance (O.D.at 470nm), odor, specific gravity and moisture content. For the observance which show the (O.D. at 470nm) of deteriorated oil due to storage fungi infection the transparency of the oil are changed and varied, and there is a noticeable difference in oil absorbance (470 nm), with infection of *Fusarium semitectum* (0.081) and *Stemphylium* spp. (0.099), also, the Odor was rancid with both of them, these results may due to the secondary metabolites. The specific gravity of the tested oils is varied with *Fusarium semitectum* (0.308) and *Stemphylium* spp. (0.338).

Comparing the moisture content the highest content was 8.0 and 7.0 in case of *Fusarium semitectum* and *Stemphylium* sp., respectively. The obtained results come to other results reported by [12], [13] and [14] , they suggested that the changes in oil colour may be due to pigments synthesized by invading fungi like *Aspergillus* spp. Also, they reported that the extracted sunflower oils are of a great significance as a vegetarian edible oil free of high content of cholesterols and fats [13], [14].

**Table ( 3 ). The Physical changes in sunflower oil due to storage fungi activity.**

| Fungi | Moisture content | Oil colour | Specific gravity | Odor | Absorbance (470 nm) |
|---|---|---|---|---|---|
| *Aspergillus flavus* | *3.0 | Pale yellow | 0.299 | Normal | 0.113 |
| *Aspergillus niger* | 1.0 | Yellow | 0.344 | Normal | 0.122 |
| *Alternaria alternata* | 3.0 | Bright yellow | 0.280 | Normal | 0.114 |
| *Curvularia lunata* | 3.0 | Yellow | 0.333 | Normal | 0.124 |
| *Fusarium moniliforme* | 1.0 | Yellow | 0.290 | Normal | 0.111 |
| *Fusarium oxysporum* | 4.0 | Pale yellow | 0.342 | Normal | 0.128 |
| *Fusarium semitectum* | 8.0 | Pale yellow | 0.308 | Rancid | 0.081 |
| *Penicillum digitatum* | 5.0 | Pale yellow | 0.289 | Specific | 0.137 |
| *Stemphylium* sp. | 7.0 | Pale Yellow | 0.338 | Rancid | 0.099 |
| *Trichoderma* spp. | 2.0 | Yellow | 0.296 | Normal | 0.144 |
| Check | 2.0 | Yellow | 0.234 | Normal | 0.165 |

***Data were investigated for each fungus individually relative to check treatment.**

For the chemical changes as shown in Fig.(3), sunflower oils due to the deterioration process caused by storage seed-borne fungi data in Table (4), show that, the *Aspergillus flavus* increased the free fatty acid content (3.820) and *Curvularia lunata* ( 3.675), on the other hand, Free fatty acid content due to *Alternaria alternata* was decreased to (1.512 %) also, with *Fusarium semitectum* (1.675 %) ,these results are similar to those reported by [15], [16] and [17].

The highest percentage of the saponification number (s.n.) of deteriorated sunflower oils was calculated and results are summarized in Table (4) *Aspergillus niger* increased the s.n. to 344.02, while *Curvularia lunata* decreased the s.n. to 186.45.Investigating the iodine numbers show that *Fusarium semitectum* (150.78) and *Curvularia lunata* (138.49) recorded the highest iodine number , whereas other fungi results in low level like *Fusarium oxysporum* (100.14). Peroxide value was estimated relative to control and the highest was shown with *Aspergillus flavus* infection at 56 also the lowest was with *Fusarium moniliforme* (11), the peroxide value is an indicator of oil sample if its rancid or still stable with good quality . These results are in agreement with those reported by [17] and [18], both of rancidity and Saponification number reflects the quality of oil for its

validity if it is stable or deteriorated and can't be consumed for human consumption as reported [15],[16], [17]and [18].

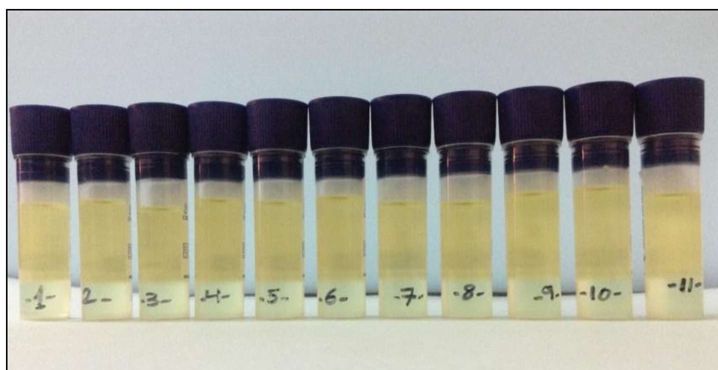


**Fig. (3). The deteriorated sunflower oils for 10 samples relative to check treatment no. 11 without inoculation.**

**Table (4). The Chemical changes in sunflower oil due to storage fungi activity .**

| Fungi | Peroxide value (mEq/kg) | Iodine Number | Saponification Number | Free Fatty acids (%) |
|---|---|---|---|---|
| *Aspergillus flavus* | *56 | 115.23 | 190.41 | 3.820 |
| *Aspergillus niger* | 13 | 113.78 | 344.02 | 2.980 |
| *Alternaria alternata* | 48 | 106.21 | 210.40 | 1.512 |
| *Curvularia lunata* | 16 | 138.49 | 186.45 | 3.675 |
| *Fusarium moniliforme* | 11 | 116.90 | 288.02 | 3.245 |
| *Fusarium oxysporum* | 36 | 100.14 | 265.07 | 3.657 |
| *Fusarium semitectum* | 17 | 150.78 | 198.54 | 1.675 |
| *Penicillum digitatum* | 18 | 116.74 | 255.08 | 2.987 |
| *Stemphylium* sp. | 54 | 103.32 | 223.60 | 2.346 |
| *Trichoderma* spp. | 14 | 106.78 | 230.04 | 3.547 |
| Check | 16 | 112.56 | 199.65 | 1.150 |

***Data were investigated for each fungus individually relative to control treatment.**

## IV. Conclusion

Sunflower (*Helianthus annuus* L.), considered a commercial oil crop all over the world, the crop is widely cultivated in Egypt and in many countries all over the world. Sunflower is particularly used for production of edible oils as well as for seed consumption. The crop is attacked by numerous seed mycoflora and these pathogens may affect the crop resulting in a reduction of the seed quantity and quality. The direct impact of storage fungi on the economical part of the plant (seed) need further studies for studying the different effects of storage fungi on sunflower oil in order to increase oil yield and crop quality for human consumption and food industries.

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